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(54) (Title of the Invention) α-HYDROXYGLYCINAMIDE DERIVATIVES AND PREPARATION THEREOF

(57) (Abstract)

(Object) To obtain an amidation agent suitable for amidation of C-terminal carboxyl groups of amino acids or peptides, without racemization.

(Structure) An α-hydroxyglycinamide derivative represented by the following formula (I)

(I)

(wherein R^1 is a hydrogen atom, a lower alkyl group, a lower alkenyl group, a lower alkynyl group, or a silyl group substituted with an alkyl group or an alkyl group and an aromatic group; R^2 is a hydrogen atom or an amino protecting group), a salt thereof, a method for preparation thereof, and a method for amidating C-terminal carboxyl groups of amino acids, peptides, or derivatives thereof by using this compound.

(Scope of Patent Claims)

(Claim 1) An α-hydroxyglycinamide derivative represented by the following formula (I)

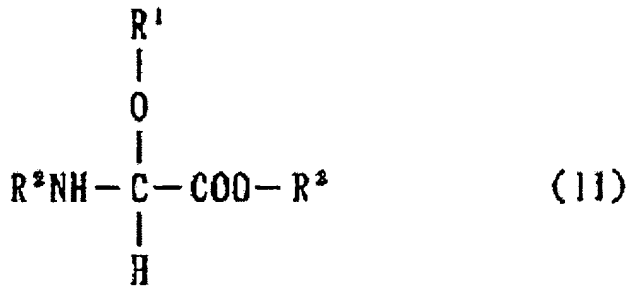
(Formula 1)

(I)

(wherein R^1 is a hydrogen atom, a lower alkyl group, a lower alkenyl group, a lower alkynyl group, a benzyl group, or a silyl group substituted with an alkyl group or an alkyl group and an aromatic group; R^2 is a hydrogen atom or an amino protecting group) and a salt thereof.

(Claim 2) A method for the preparation of the α-hydroxyglycinamide derivative or salt thereof described in Claim 1, characterized in that an α-hydroxyglycine derivative represented by the following formula (II)

(Formula 2)



(wherein R^1 and R^2 are defined in Formula (I); R^3 is a hydrogen atom or a carboxyl protecting group) is treated with ammonia in a solvent, the amino protecting group is removed if desired, and the compound obtained is further converted into a salt thereof if desired.

(Claim 3) A method for the preparation of a C-terminal amidated peptide, characterized in that an amino acid or a peptide or a derivative thereof is reacted with the α-hydroxyglycine derivative described in Claim 1.

(Detailed Description of the Invention)

(0001)
(Industrial Field of the Invention) The present invention relates to an α-hydroxyglycine derivative and a salt thereof, this compound being suitable for the preparation of C-terminal amidated peptides.

(0002)
(Prior Art) A variety of peptide physiologically active substances requiring amidation of C-terminal for physiological activity are known. Examples of such physiologically active peptides include melanotropin release-inhibiting hormones (Pro-Leu-Gly-NH_2) [R.M.G. Nair et al., Biochem. Biophys. Res. Commun., 43, 1376 (1971); M. E. Celis et al., Pro. Nat. Acad. Sci., USA, 68, 1428 (1971), thyrotropin-releasing hormones (Pyro-Glu-His-Pro-NH_2) [K. Folkers et al., Biochem. Biophys. Res. Commun., 37, 123, 705 (1969); Endocrinol., 86, 1143 (1970); R.Burgus et al., Compt. Rend. Acad. Sci., 269, 1870 (1969); Nature, 226, 321 (1970), and the like.

(0003) The following methods are known for the preparation of such physiologically active C-terminal amidated peptides: a method comprising amidation of carboxyl group of peptide C-terminal [Zhang Hongliang et al., Yiyao Gongye, 3, 3 (1983); Chem. Abst. 99, 639 (1983); Vlassa M., Rev. Roum. Chim. 21, 455 (1976); Rivaille Pierre et al., Helv. Chim. Acta, 54, 355 (1971); Folkers Karl et al., J. Med. Chem., 14, 475-6 (1971); Beyerman, H. C. et al., Rect. Trav. Chim. Pays-Bus, 90, 791 (1971); Folkers Karl et al., Chem. Abst., 79, 459 (1973)], a method based on chemical or enzyme condensation of glycinamide or prolinamide [Muro Tetsuo et al., Agric. Biol. Chem., 51, 1207 (1987); Flouret George, J. Med. Chem., 13, 843 (1970); Fluoret George, Chem. Abst., 75, 246 (1971); Wissmann Hans et al., Chem. Abst., 76, 449 (1972); H. Chitoshi et al., Biochem. Biophys. Res. Commun., 60, 1345 (1974); Kurath P. et al., Helv. Chim.

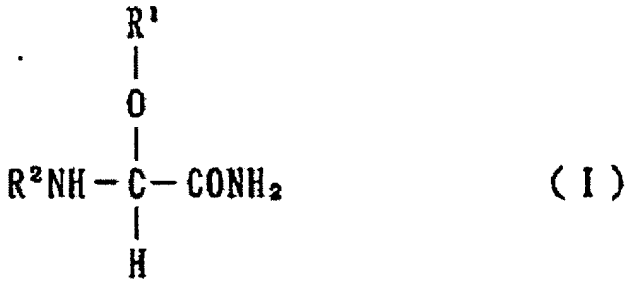
Acta., 56, 1656 (1973); Bienert Michael et al., Chem. Abst., 86, 455 (1976); Bienert M., Pharmazie 32, 397 (1977)]

(0004) However, in the above-described chemical methods, a reaction is conducted between a protected C-terminal carboxyl group and ammonia gas, and in this case amidation of C-terminal cannot be conducted without racemization because such a reaction enhances racemization of optically active peptides.

(0005)
(Problems to be Resolved by the Invention) Accordingly it is an object of the present invention to provide novel means for C-terminal amidation of peptides without racemization.

(0006)
(Means for Resolving the Problems) The inventors have conducted a comprehensive study aimed at the resolution of the above-described problems and have discovered that using a compound represented by the following formula (I)

(Formula 3)

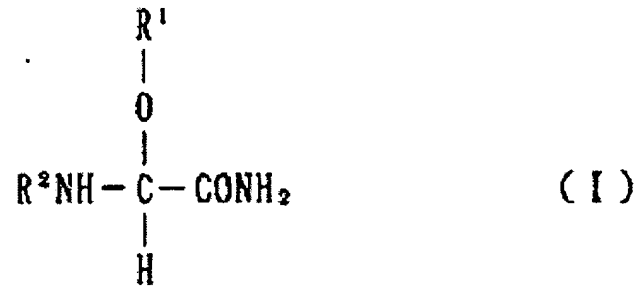


(I)

as an amino group donor makes it possible to conduct amidation, without racemization, of carboxyl groups in a peptide in which the C-terminal carboxyl groups are not protected.

(0007) Accordingly, the present invention provides an α-hydroxyglycinamide derivative represented by the following formula (I)

(Formula 4)

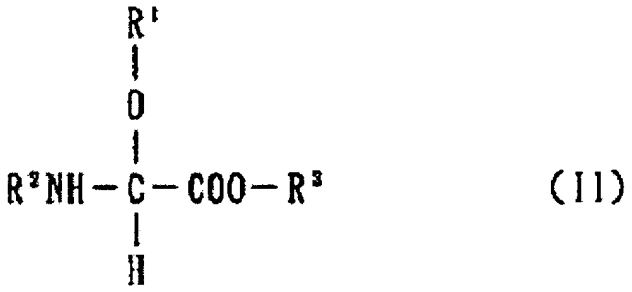


(I)

(wherein R^1 is a hydrogen atom, a lower alkyl group, a lower alkenyl group, a lower alkynyl group, a benzyl group, or a silyl group substituted with an alkyl group or an alkyl group and an aromatic group; R^2 is a hydrogen atom or an amino protecting group) and a salt thereof.

(0008) The present invention also provides a method for the preparation of the α-hydroxyglycinamide derivative or salt thereof described hereinabove, characterized in that an α-hydroxyglycine derivative represented by the following formula (II)

(Formula 5)



(II)

(wherein R^1 and R^2 are defined in Formula (I); R^3 is a hydrogen atom or a carboxyl protecting group) is treated with ammonia in a solvent, the amino protecting group is removed if desired, and the compound obtained is further converted into a salt thereof if desired.

(0009) The present invention also provides a method for the preparation of a C-terminal amidated peptide, characterized in that an amino acid or a peptide or a derivative thereof is reacted with the α-hydroxyglycine derivative described hereinabove.

(0010)
(Detailed Explanation) In accordance with the present invention, the lower alkyl group represented by reference symbol R_1 is an alkyl group containing no more than 6, preferably no more than 4 carbon atoms. Examples of such groups include methyl group, ethyl group, n-propyl group, isopropyl group, n-butyl group, isobutyl group, tert-butyl group, pentyl group that may be branched, and hexyl group that may be branched.

(0011) The lower alkenyl group represented by reference symbol R_1 is an alkenyl group containing no more than 6, preferably no more than 4 carbon atoms. Examples of such groups include ethenyl group, allyl group, and butenyl group having a double bond in any position. The lower alkynyl group represented by reference symbol R_1 is an alkynyl group containing no more than 6, preferably no more than 4 carbon atoms. Examples of such groups include ethynyl group and the like.

(0012) The silyl group substituted with a lower alkyl group, which is represented by reference symbol R_1, is a silyl group substituted with 1 to 3 lower alkyl groups. The lower alkyl substituents used in this case are any of the lower alkyl groups described hereinabove with reference to R_1 or combinations thereof. The silyl group substituted with a lower alkyl group is preferably a tert-butyldimethylsilyl group. The silyl group substituted with an alkyl and an

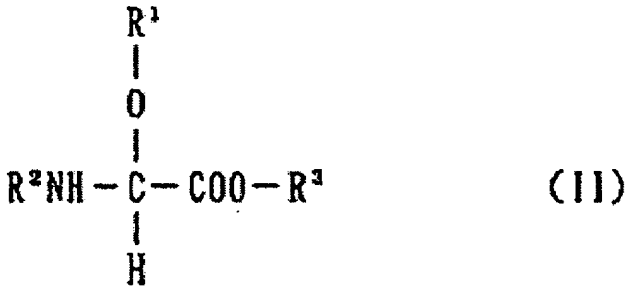
aromatic group is a silyl group substituted with the above-described alkyl group and phenyl group, for example, tert-butyldiphenylsilyl group.

(0013) Protecting groups that have been used in the field of amino acid or peptide chemistry can be used as the amino protecting group represented by R_2. Examples of such groups include oxycarbonyl-type protecting groups, for example, benzyloxycarbonyl (Cbz-), p-methoxybenzyloxycarbonyl [Z(OMe)-], tert-butoxycarbonyl (Boc-), or 2-biphenylisopropoxycarbonyl (Bpoc-), and the like; acyl protecting groups, for example, HCO-, phthalate group (Pht-), or o-nitrophenylthio group (Nps-), and the like; and alkyl protecting groups, for example, triphenylmethyl group (Trt-), and the like.

(0014) Salts of the α-hydroxyglycinamide derivative in accordance with the present invention are acid-added salts, for example, inorganic salts such as hydrohalides, e.g. hydrofluorides, hydrochlorides, hydrobromides, nitrates, sulfates, or phosphates, or organic acid salts such as fumarates, acetates, and the like.

(0015) The compounds represented by formula (I) in accordance with the present invention can be prepared by treating an α-hydroxyglycine derivative represented by the following formula (II)

(Formula 6)



(II)

(wherein R^1 and R^2 are defined in Formula (I); R^3 is a hydrogen atom or a carboxyl protecting group) with ammonia in a solvent and optionally removing the amino protecting group.

(0016) The carbonyl protecting group R^3 is an ordinary carboxy protecting group that can be substituted with amino group by treatment with ammonia. Examples of such groups include lower alkyloxy groups, for example, methoxy group (-OMe), ethoxy group (-OEt), benzyloxy group (-OBz1), or tert-butoxy group (-OtBu), or aryloxy group, such as p-nitrophenoxy group (-ONp), and the like.

(0017) Ordinary organic solvents such as lower alcohols, for example methanol, ethanol, propanol, ethers such as methyl ethyl ether, diethyl ether, isopropyl ether, and the like can be used as the solvents for the reaction. The reaction can be conducted by dissolving the compound represented by formula (II) in the above-mentioned solvent and blowing ammonia under reduced, normal, or increased pressure at a temperature, for example, from -78°C to 40°C, preferably from 0°C to 25°C, e.g. at room temperature.

(0018) This reaction makes it possible to obtain the compound (I) in accordance with the present invention, in which R^2 is an amino protecting group. In order to remove the amino protecting group R^2 from this compound and to obtain the compound (I) in accordance with the present invention, in which R^2 is hydrogen, usual deprotecting treatment may be conducted according to the type of the amino protecting group R^2. For example, when the protecting group R^2 is benzyloxycarbonyl, P-methoxybenzyloxycarbonyl, and the like, deprotecting can be carried out by conducting treatment with hydrogen gas in the presence of a hydrogenation catalyst, for example, palladium/carbon or the like. Furthermore, when the protecting group R^2 is tert-butoxycarbonyl, deprotecting can be conducted with hydrochloric acid – dioxane. A salt of the compound (I) in accordance with the present invention can be produced, for example, by conducting the above-described deprotecting treatment in the presence of an acid such as hydrochloric acid.

(0019) A compound among the intermediate compounds (II), in which R^1 is not a hydrogen atom, can be produced, for example, by the following two methods. With the first method, it can be produced by introducing R^1 other than hydrogen into the compound among the compounds represented by formula (II), in which R^1 is hydrogen. The introduction of the group R^1 other than hydrogen can be conducted with the respective functional derivative of the group, for example, a halogen derivative. For example, for introducing a lower alkyl substituted silyl group, a halide of silyl group can be used, for example, tert-butyldimethylsilyl chloride can be used for introducing a tert-butoxydimethylsilyl group. This reaction can be conducted at a temperature of from 0°C to 30°C in a solvent such as dimethylformamide.

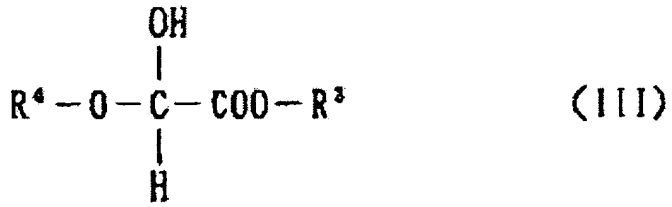
(0020) Furthermore, in order to introduce a lower alkenyl or lower alkynyl group, a halogen derivative of alkene or alkyl respectively can be used. For example, an allyl group can be introduced by using an allyl halide such as allyl iodide in the presence of a catalyst such as silver oxide. This reaction can be conducted at a temperature from -10 to 50°C, preferably from 0°C to 25°C, in a solvent such as dimethylformamide.

(0021) With the other method for producing the intermediate compound (II) in which R^1 is not hydrogen, the compound represented by formula (II) in which both R^1 and R^2 are hydrogen atoms is treated with thionyl chloride by using a lower alcohol, for example methanol or ethanol as a solvent. In this case, a compound represented by formula (II) in which R^1 and R^2 are the same lower alkyl group corresponding to the lower alcohol solvent can be obtained. The reaction can be conducted at a temperature from -10°C to 40°C, preferably from 0°C to 25°C.

(0022) The intermediate represented by formula (II) in which R^1 is hydrogen can be produced, for example, by the following two methods. With the first method, it can be obtained by reacting glyceraldehydes CHO-COOH with an amine R^2NH_2 protected with amino protecting group R^2. This reaction can be conducted at a temperature of 20°C to 75°C in a solvent such as acetone, ether, and the like, for example, by a method described in US Patent No. 3,668,121 issued to Philip X. Masciantonio et al., and by Stanlen D. Young et al., J. Am. Chem. Soc. 111, 1933 (1989). In this case, a compound represented by formula (II) in which both the R^1 and the R^3 are hydrogen atoms can be obtained.

(0023) With the other method for the preparation of the intermediate represented by formula (II) in which R^1 is hydrogen, a compound represented by the following formula (III):

(Formula 7)



(III)

(wherein R^3 is defined as described with reference to formula (II), and R^4 is a lower alkyl group) is reacted with an amine R^2NH_2 protected with amino protecting group R^2. This reaction can be conducted in a solvent such as tetrahydrofuran at a temperature of 20°C to 80°C, for example, at the reflux temperature of the solvent used. The lower alkyl group R^4 is defined as the lower alkyl group R^1.

(0024) Amidation of C-terminal of a peptide or amino acid derivative can be conducted by the hydroxyglycine derivative in accordance with the present invention, which is obtained in the above-described manner and represented by formula (I). For this purpose, the compound represented by formula (I) and a peptide in which C-terminal is not protected are reacted in an aprotic solvent, for example, dimethylformamide (DMF), phosphoric hexamethyltriamide (HMPA), dimethylsulfoxide (DMSO), and the like, in the presence of a dehydrogenation condensation agent, for example, dicyclohexylcarbodiimide (DCC), water-soluble carbodiimide (WSCD). The reaction is preferably conducted within a temperature range from -50°C to room temperature.

(0025) This method can be used for the preparation of C-terminal amidated physiologically active peptides, for example, melanotropin release-inhibiting hormones, thyrotropin-releasing hormones, human calcitonin, salmon calcitonin, and the like.

(0026) The present invention will be described herein below in greater detail based on examples thereof.

Example 1

1-1

α-Hydroxy-N-tert-butoxycarbonylglycine methyl ester (4.11 g, 20 mmol) and imidazole were dissolved in DMF at room temperature and cooled to a temperature of 0°C. Then chlorinated tert-butyldimethylsilyl was added to the solution at this temperature and the components were stirred for 10 min. The solution was returned to room temperature and stirring was continued for 1 hour. Then, saturated brine was added and extraction was conducted with ethyl acetate. The organic layer was dried with anhydrous magnesium sulfate and the solvent was distilled off.

(0027) The oily substance thus obtained was dissolved in ethanol (50 mL) and excess ammonia was blown into the solution at a temperature of 0°C.

The excess ammonia was then removed under reduced pressure and ethanol was distilled off. The crude product thus obtained was purified by silica gel column chromatography and α-tert-butyldimethylsilyloxy-N-tert-butoxycarbonylglycinamide (6.10 g, quant.) was obtained. ^{1}HNHR (sic) δ($CDCl_3$) 0.16(s, 3H), 0.21(s, 3H), 0.92(s, 9H), 5.46(d, 1H, J=9Hz), 5.63(d, 1H, J=9Hz), 6.22-6.82 (br, 2H).

(0028) 1-2
The α-hydroxy-N-tert-butoxycarbonylglycine methyl ester that was a starting substance in 1-1 above was prepared in the manner as follows. tert-Butyl carbamate(2.83 g, 23.6 mmol) and glyoxylic acid monohydrate (2.02 g, 21.5 mmol) were dissolved in acetone (50 mL) and refluxed overnight. The solution was then cooled to a temperature of 0°C and treated with excess diazomethane-ether solution at this temperature. The solvent was then distilled off.

(0029) Saturated brine was then added, extraction was conducted with chloroform, the organic layer was dried with anhydrous magnesium sulfate and the solvent was distilled off. The crude product thus obtained was purified by silica gel column chromatography and α-hydroxy-N-tert-butoxycarbonylglycine methyl ester (2.56 g, 58%) was obtained.
^{1}HNMR δ($CDCl_3$) 1.46 (s, 9H), 1.65 (br s, 1H), 3.84 (s, 3H), 5.27-5.52 (br, 1H), 5.59-5.90 (br, 1H)
IR(NaCl) 1755(s), 1690(s), 1528(s)cm^{-1}.

(0030) 1-3
The α-hydroxy-N-tert-butoxycarbonylglycine methyl ester that was a starting substance in 1-1 above was prepared by a method other than that of 1-2. tert-Butyl carbamate (11.35 g, 95.0 mmol) and 1-hydroxy-1-methoxyacetic acid methyl ester (14.35 g, 119.5 mmol) were dissolved in anhydrous THF (50 mL) and refluxed overnight. The temperature was then returned to room temperature, 1-hydroxy-1-methoxyacetic acid methyl ester (1.15 g, 9.6 mmol) was then added and the components were further refluxed for 8 h. The reaction liquid was allowed to sit until the temperature returned to room temperature and the solvent was then distilled off. The crude product thus obtained was recrystallized from a chloroform-hexane solution and pure α-hydroxy-N-tert-butoxycarbonylglycine methyl ester (16.42 g, 84%) was obtained.

(0031) Example 2. The α-hydroxy-N-tert-butoxycarbonylglycine methyl ester (1.21 g, 5.9 mmol) obtained in 1-2 or 1-3 above was dissolved in DMF (10 mL), and then silver oxide (1.04 g, 4.5 mmol) and benzene iodide (1.99 g, 9.1 mmol) were added at room temperature. The components were stirred overnight at room temperature, the precipitate was filtered, water was added to the mother liquor, and extraction was conducted with ethyl acetate. The extracted solution was dried with anhydrous magnesium sulfate, then the solvent was distilled off and crude purification was conducted with silica gel column chromatography.

(0032) The oily substance thus obtained was dissolved in ethanol (50 mL) and excess ammonia was blown into the solution at a temperature of 0°C. The excess ammonia was then removed under reduced pressure and the solvent was distilled off. The crude product thus obtained was purified by silica gel column chromatography and α-benzyloxy-N-tert-butoxycarbonylglycinamide (0.397 g, 22%) was obtained.
m.p. 115-120°C

^{1}HNMR δ($CDCl_3$) 1.44 (s, 9H), 4.61 (d, 1H, J=11.3Hz), 4.79 (d, 1H, J=11.3Hz), 5.4 (d, 1H, J=9.0Hz), 5.75 (brd, 1H, J=9.0Hz), 6.00 (br, 1H), 6.52 (br, 1H), 7.35 (s, 5H)
IR(NaCl) 1698(s), 1664(s), 1502(s), 732(m), 695(m) cm^{-1}.
Analytical values for elements ($C_{14}H_{20}O_4N_2$): Calcd. C:59.99, H:7.19, N:9.99
Obsd. C:59.94, H:7.33, N:10.28

(0033) Example 3. The α-hydroxy-N-tert-buthoxycarbonylglycinemethyl ester (2.07 g, 10.1 mmol) prepared according to 1-2 or 1-3 above was dissolved in DMF (20 mL), and silver oxide (1.39 g, 6.0 mmol) and allyl iodide (1.2 mL, 12.9 mmol) were added at room temperature. After overnight stirring at room temperature, the precipitate was filtered out, water was added to the mother liquor, and extraction with ethyl acetate was conducted. The extracted solution was dried with anhydrous magnesium sulfate, then the solvent was distilled off, and an aqueous solution of sodium thiosulfate was added, followed by extraction with ethyl acetate and removal of iodine as a reaction byproduct.

(0034) The oily substance thus obtained was dissolved in ethanol, excess ammonia was blown into the solution at a temperature of 0°C, the excess ammonia was thereafter removed under reduced pressure, and the solvent was distilled off. The crude produce obtained was purified with silica gel column chromatography to obtain α-allyloxy-N-tert-butoxycarbonylglycinamide (0.625 g, 27%).
^{1}HNMR δ($CDCl_3$) 1.45 (s, 9H), 4.14 (dd, 2H, J=7.2, 1.8Hz), 5.11-5.56 (m, 3H), 5.70-6.20 (m, 2H), 6.33-7.01 (m, 2H)
IR($CDCl_3$) 2975(w), 1705(s, br), 1498(m), 990(sh.w) cm^{-1}

(0035) Example 4
4-1
α-Hydroxy-N-benzyloxycarbonylglycine (4.44 g, 19.7 mmol) was dissolved in methanol (20 mL). Thionyl chloride (2.9 mL, 40.0 mmol) was dropwise added to the solution at a temperature of 0°C, and stirring was conducted for 30 minutes at this temperature and then for 2 hours at room temperature. The solvent was then distilled off and the crude product obtained was dissolved in methanol (50 mL). The solution was cooled to 0°C, and excess ammonia was blown therein.

(0036) Upon completion of the reaction, the excess ammonia was removed under reduced pressure, the solvent was distilled off, and the white crystals obtained were purified with silica gel column chromatography to obtain α-methoxy-N-benzyloxycarbonylglycinamide (3.42 g, 73%).

m.p. 110-112°C
^{1}HNMR δ($CDCl_3$) 3.44 (s, 3H), 5.16 (s, 2H), 5.31 (d, 1H, J=8.8Hz), 5.45-5.98 (br, 2H), 6.28-6.68 (br, 1H), 7.36 (s, 5H)
IR(NaCl) 1680(s. br), 1540(s), 1520(s), 860(m), 700(m) cm^{-1}
Analytical values of elements ($C_{11}H_{14}O_4N_2$); Calcd. C:55.46, H:5.92, N:11.76
Obsd. C:55.70, H:5.94, N:11.58.

(0037) 4-2
The α-hydroxy-N-benzyloxycarbonylglycine that was the starting material in 1-4 above was prepared in the manner as follows. Benzyl carbamate (30.24 g, 0.2 mol) and glyoxylic acid monohydrate (20.26 g, 0.22 mol) were dissolved in diethyl ether (200 mL) and the solution was stirred overnight at room temperature. The crystals produced were filtered and then washed with ether to obtain pure α-hydroxy-N-benzyloxycarbonylglycine (33.78 g, 75%).
m.p. 200-205°C
^{1}HNMR δ(CD_3OD) 5.12 (s, 2H), 5.40 (s, 1H), 7.34 (s, 5H)

(0038) Example 5. The α-hydroxy-N-benzyloxycarbonylglycine (2.26 g, 10.0 mmol) produced according to 4-2 above was dissolved in ethanol (20 mL). Thionyl chloride (2 mL, 27.4 mmol) was dropwise added to the solution at a temperature of -10°C, and stirring was conducted overnight at room temperature. The solvent was then distilled off and the crude product thus obtained was purified with silica gel column chromatography to obtain α-ethoxy-N-benzyloxycarbonylglycine ethyl ester (2.81 g, quant.).
m.p. 66-68°C
^{1}HNMR δ($CDCL_3$) 1.22 (t, 3H, J=7.2 Hz), 1.30 (t, 3H, J=7.2 Hz), 3.70 (q, 2H, J=7.2 Hz), 4.24(q, 2H, J=7.2 Hz), 5.15 (s, 2H), 5.33 (d, 1H, J=9.7 Hz), 5.93 (brd, 1H, J=9.7 Hz), 7.35 (s, 5H).
IR(NaCl) 1740(s), 1700(s), 1540(s), 760(m), 700(m) cm^{-1}
Analytical values of elements ($C_{14}H_{19}O_5N$); Calcd. C:59.78, H:6.81, N:4.98
Obsd. C:60.03, H:6.88, N:4.89.

(0039) Example 6. The α-hydroxy-N-benzyloxycarbonylglycine (2.26 g, 10.0 mmol) produced according to 4-2 above was dissolved in isopropyl alcohol (20 mL). Thionyl chloride (2 mL, 27.4 mmol) was dropwise added to the solution at a temperature of -10°C, and stirring was conducted overnight at room temperature. The solvent was then distilled off and the crude product thus obtained was purified with silica gel column chromatography to obtain α-isopropoxy-N-benzyloxycarbonylglycine isopropyl ester (3.10 g, quant.).
^{1}HNMR δ($CDCL_3$) 1.16-1.37 (m, 12H), 3.87-4.22 (m, 1H), 4.57-5.20 (m, 1H), 5.14 (s, 2H), 5.33 (d, 1H, J=9.7 Hz), 5.93 (brd, 1H, J=9.7 Hz), 7.35 (s, 5H).
IR(Neat) 1728(s, br), 1508(m), 740(m) cm^{-1}.

(0040) Example 7. The α-ethoxy-N-benzyloxycarbonylglycine ethyl ester (2.29 g, 8.1 mmol) produced according to Example 5 was dissolved in ethanol (80 mL) and cooled to 0°C. Excess ammonia was then blown into the solution at this temperature. Upon completion of the reaction, the excess ammonia was removed under reduced pressure, the solvent was distilled off, and the white crystals thus obtained were washed with a hexane - ethyl acetate mixed solution to obtain pure α-ethoxy-N-benzyloxycarbonylglycinamide (1.51 g, 77%).

m.p 119-121°C
^{1}HNMR δ($CDCL_3$) 1.23 (t, 3H, J=7.1 Hz), 3.50-3.90 (m, 2H), 5.14 (s, 2H), 5.37 (d, 1H, J=9.0 Hz), 5.65-5.96 (br, 2H), 6.41-6.71 (br, 1H), 7.35 (s, 5H).
IR(NaCl) 1680(s), 1664(s), 1542(m), 1524(m), 760(w), 740(w), 700(m) cm^{-1}
Analytical values of elements ($C_{12}H_{16}O_4N_2$); Calcd. C:57.13, H:6.39, N:11.10
Obsd. C:57.09, H:6.34, N:11.37.

(0041) Example 8. The α-isopropoxy-N-benzyloxycarbonylglycine isopropyl ester (2.48 g, 8.0 mmol) produced according to Example 6 was dissolved in ethanol (40 mL) and cooled to 0°C. Then, excess ammonia was blown into the solution for 5 hours at this temperature and stirring was further conducted for 2 days in the ammonia saturated state. Upon completion of the reaction, the excess ammonia was removed under reduced pressure, the solvent was distilled off, and the white crystals thus obtained were washed with a hexane - ethyl acetate mixed solution to obtain pure α-isopropoxy-N-benzyloxycarbonylglycinamide (1.64 g, 77%).
m.p 111-113°C
^{1}HNMR δ($CDCL_3$) 1.18 (d, 3H, J=4.4 Hz), 1.25 (d, 3H, J=4.4 Hz), 3.81-4.20(m, 1H), 5.15 (s, 2H), 5.44 (d, 1H, J=9.0Hz), 5.53-5.86 (br, 2H), 6.37-6.73 (br, 1H), 7.35 (s, 5H).
IR(NaCl) 1668(s), 1660(s), 1538(m), 1530(m), 760(w), 740(w), 700(m) cm^{-1}
Analytical values of elements ($C_{13}H_{18}O_4N_2$); Calcd. C:58.63, H:6.81, N:10.52
Obsd. C:58.60, H:6.82, N:10.54.

(0042) Example 9. The α-tert-butyldimethylsilyloxy-N-tert-buthoxycarbonylglycinamide (5.08 g, 16.7 mmol) produced according to (1-1) of Example 1 was dissolved in dioxane (10 mL) and cooled to 0°C. Then, a 4N hydrochloric acid – dioxane solution (17 mL) was added and stirring was conducted for 1 hour at this temperature.

In order to complete the reaction, a 4N hydrochloric acid – dioxane solution was further added, the temperature was raised to room temperature and stirring was conducted for 1 hour. Diethyl ether was then added to the solution, as large an amount of the product as possible was precipitated, filtered, and washed with ether. The precipitate was then dried under reduced pressure to obtain pure α-hydroxyglycinamide hydrochloride (1.86 g, 88%).

^{1}HNMR δ(DMSO-d_6) 4.99 (br sd, 1H), 7.62-8.03 (br, 2H), 8.32-8.85 (br, 3H)
IR (KBr) 1686 (s), 1581(m), 1546 (m), 1477 (s), 843 (m) cm^{-1}

(0043) Example 10. The α-methoxy-N-benzyloxycarbonylglycinamide (0.24 g, 1.0 mmol) prepared according to Example 4 (4-1) was dissolved in methanol, 12N hydrochloric acid (0.1 mL) and palladium-carbon (50 mg) were added to the solution at room temperature, and stirring was conducted for 30 minutes under hydrogen atmosphere. The palladium-carbon was then filtered out and the solvent of the mother liquor was distilled off to obtain α-methoxyglycinamide hydrochloride (0.14 g, quant) as a target product.
^{1}HNMR δ(CD_3OD) 3.35 (s, 3H), 5.01 (s., 1H)
^{13}CNMR δ(CD_3OD) 42.1, 84.3 (d, J= 159.8 Hz), 170.3.

(0044) Example 11. Amidation of amino acid
The α-hydroxyglycinamide hydrochloride (72.6 mg, 0.57 mmol) prepared according to Example 9, N-tert-butoxycarbonyl-phenylalanine (125.8 mg, 0.48 mmol), and N-hydroxybenzotriazole (78 mg, 0.58 mmol) were dissolved in dimethylformamide (4 mL), and the solution was cooled to a temperature of -10°C. Then, water-soluble carbodiimide (0.1 mL, 0.55 mmol) was dropwise added, the temperature was raised to 0°C, and stirring was conducted for 2 hours.

(0045) Then water was added, extraction with chloroform was conducted, the organic layer was dried with anhydrous magnesium sulfate, and the solvent was distilled off. The crude product thus obtained was purified by using TLC for fractionation and N-tert-butoxycarbonyl-phenylalaninamide (38 mg, 30%) was obtained as a target product.

m.p. 142-149°C.
$[\alpha]_D = +16.5°$ (EtOH, C=1.17)
^{1}HNMR δ ($CDCl_3$) 1.40 (s, 2H), 3.07 (d, 2H, J = 6.8 Hz), 4.36 (dt, 1H, J = 7.9, 6.9), 4.92-5.20 (brd, 1H), 5.32-5.59 (br, 1H), 5.59-5.93 (br, 1H), 7.25 (s, 5H).

(0046) Example 12. Amidation of amino acid
The α-hydroxyglycinamide hydrochloride (0.122 g, 9.6 mmol) prepared according to Example 9, N-tert-butoxycarbonyl-proline (0.169 g, 0.79 mmol), and N-hydroxybenzotriazole (0.129 g, 0.95 mmol) were dissolved in dimethylformamide (5 mL), and the solution was cooled to a temperature of -10°C. Then, water-soluble carbodiimide (0.1 mL, 0.55 mmol) was dropwise added, stirring was conducted for 5 minutes at this temperature, the temperature was then raised to 0°C, and stirring was further conducted for 4 hours.

(0047) Then, brine was added, extraction was conducted with chloroform, the organic layer was dried with anhydrous magnesium sulfate, and the solvent was distilled off. The crude product thus obtained was purified by using silica gel column chromatography and N-tert-butoxycarbonyl-prolinamide (0.081 g, 48%) was obtained as a target product.
m.p. 104-106°C
$[\alpha]_D = -40.5°$ (EtOH, C=1.1)
^{1}HNMR δ($CDCl_3$) 1.40 (s, 9H), 1.65-2.48(m, 4H), 3.27-3.63 (brt, 2H), 4.18-4.41 (br, 1H), 5.88-7.02 (br, 2H)

(0048) Example 13. Amidation of tripeptide
The α-hydroxyglycinamide hydrochloride (0.58 g, 4.6 mmol) prepared according to Example 9, N-tert-butoxycarbonyl-L-prolyl-L-leucyl-glycine (1.37 g, 4.2 mmol), and N-hydroxybenzotriazole (0.63 g, 4.7 mmol) were dissolved in dimethylformamide (15 mL), and the solution was cooled to a temperature of -10°C. Then, water-soluble carbodiimide (0.85 mL, 4.6 mmol) was dropwise added, stirring was conducted for 20 minutes at this temperature, the temperature was then raised to 0°C, and stirring was further conducted overnight.

(0049) Then, brine was added, extraction was conducted with chloroform, the organic layer was dried with anhydrous magnesium sulfate, and the solvent was distilled off. The crude product thus obtained was purified by using silica gel column chromatography and N-tert-butoxycarbonyl-L-prolyl-L-leucyl-glycinamide (0.726 g, 53%) was obtained as a target product.

m.p. 119-121°C
$[\alpha]_D$ = -51.0° (DMF, C=0.73)
^{1}HNMR δ($CDCl_3$) 0.91 (d, 3H, J=5.4 Hz), 0.95(d, 3H, J=5.4 Hz), 1.46 (s, 9H), 1.20-2.45 (m, 7H), 3.45 (t, 2H, J=6.6 Hz), 3.91 (brd, 2H, J = 5.93 Hz), 4.08-4.53 (m. 2H), 5.78 (bs, 1H), 6.76 (bs, 1H), 7.03 (bs, 1H)

(0050) Example 14. Amidation of amino acid
The α-methoxyglycinamide hydrochloride (70 mg, 0.5 mmol) prepared according to Example 10, N-tert-butoxycarbonyl-phenylalanine (106 mg, 0.4 mmol), and N-hydroxybenzotriazole (69 mg, 0.5 mmol) were dissolved in dimethylformamide (2 mL), and the solution was cooled to a temperature of -10°C. Then, water-soluble carbodiimide (0.1 mL, 0.55 mmol) was dropwise added, stirring was conducted for 20 minutes at this temperature, the temperature was then raised to 0°C, and stirring was further conducted for 2.5 hours. Then, brine was added, extraction was conducted with chloroform, the organic layer was dried with anhydrous magnesium sulfate, and the solvent was distilled off. The crude product thus obtained was purified using silica gel column chromatography and N-tert-butoxycarbonyl-phenylalaninamide (37 mg, 35%) was obtained as a target product.

(0051) Example 15. α-Hydroxy-N-tert-butoxycarbonylglycine methyl ester (1.03 g, 5.0 mmol) and imidazole (0.41 g, 6.1 mmol) were dissolved in dimethylformamide (3 mL) and the solution was then cooled to a temperature of -10°C. Tert-Butyldiphenylsilyl chloride (TBDPSCl) was added to the solution and the mixture was stirred for 2 hours at room temperature. The mixture was diluted with water and eluted with ethyl acetate. The organic phase was washed with salt water and dried with anhydrous magnesium sulfate. The solvent was then evaporated under reduced pressure.

(0052) The produced crystalline residue was dissolved in ethanol (100 mL) and ammonia was blown for 3 hours into the solution at a temperature of 0°C. Stirring for 1 hour was then conducted at a temperature of 5°C, and excess ammonia was thereafter evaporated under vacuum. Upon evaporation of the solvent, the crude product was purified with silica gel column chromatography (hexane / ethyl acetate) to obtain α-tert-butyldiphenylsilyloxy-N-tert-butoxycarbonylglycinamide (2.14 g, quant.).
^{1}HNMR d($CDCl_3$), 1.07 (s, 9H), 1.31 (s, 9H), 5.25 (d, 1H, J = 8.7 Hz), 5.44 (d, 1H, J = 8.7 Hz), 6.14 (br, 1H), 6.49 (bs, 1H), 7.26-7.55 (m.6H), 7.58-7.82 (m, 4H)
IR(NaCl) 1708(s), 1690(s), 1678(s), 1520(m), 1520(m), 1080(br, m), 740(m), 700(m) cm^{-1}.

(0053) Example 16. α-Hydroxy-N-benzyloxycarbonylglycine (1.12 g, 5.0 mmol) was dissolved in 2-propyn-1-ol (5 mL), and thionyl chloride (1.1 mL, 15 mmol) was then dropwise added at a temperature of 0°C. The mixture was stirred for 14 hours till the temperature increased from 0°C to room temperature. The solvent was removed under vacuum and the crude product thus obtained was dissolved in ethanol (30 mL). Ammonia was blown into the solution at a temperature of 0°C, and the mixture was stirred overnight at room temperature under ammonia atmosphere.

(0054) Excess ammonia was removed under vacuum and the solvent was evaporated under reduced pressure. The oily residue thus produced was purified by silica gel column

chromatography (ethyl acetate) to obtain α-(2-propyn-1-oxy)-N-benzyloxycarbonylglycinamide (1.22 g, 93%).
m.p. 80-83°C
^{1}HNMR δ($CDCl_3$) 2.46 (t, 1H, J=2.4 Hz), 4.28(d, 2H, J=2.4 Hz), 5.10 (s, 2H), 5.48 (d, 1H, J=8.8Hz), 6.48 (d, 1H, J=8.8Hz), 6.65 (bs, 2H), 7.31 (S, 5H).
IR(NaCl) 2125(w), 1704(s), 1680(s), 1522(s), 758(w), 740(m), 700(m) cm^{-1}
Analytic values of elements ($C_{13}H_{14}N_2O_4$): Calculated values C:59.54, H:5.38, N:10.68.
Measured values C:59.73, H:5.51, N:10.33

(0055) Example 17. N-tert-butoxycarbonyl-L-prolyl-L-leucyl-glycinamide (0.325 g, 1.0 mmol) was dissolved in dioxane (2 mL) and then cooled to a temperature of 0°C. Then, 4N hydrochloric acid in dioxane (3 mL) was added to the solution at this temperature and the mixture was stirred for 2.5 hours at room temperature.

(0056) Then, ether was added to the solution and the precipitate produced was collected as thoroughly as possible by an over-sedimentation method, washed with ether, and vacuum dried to obtain L-prolyl-L-leucyl-L-glycinamide HCl salt (0.265 g, 96%).
$[\alpha]_D$ = -40.9° (H_2O, C = 1.1)
^{1}HNMR δ(D_2O) 0.91 (d, 3H, J=5.8 Hz), 0.95(d, 3H, J = 5.8 Hz), 1.66 (m, 3H), 2.08 (m, 3H), 2.46 (m, 1H), 3.43 (m, triplefoid, 2H), 3.91 (s. 2H), 4.41 (m. 2H).

(0057) Example 18. α-Hydroxyglycinamide hydrochloride (38 mg, 0.3 mmol), pyroglutamyl-N^{1n}-tert-butoxycarbonyl-histidylproline triethylamine salt (55 mg, 0.1 mmol) and N-hydroxybenzotriazole (27 mg, 0.2 mmol) were dissolved in dimethylformamide (0.5 mL) and cooled to a temperature of -10°C. Then, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (36 μL, 0.2 mmol) was added to the solution and the mixture was then stirred for 2 hours at the same temperature.

(0058) After subsequent stirring for 1 hour at room temperature, the mixture was subjected to direct column chromatography by using a MPLC device. The crude product was further purified by thin-layer chromatography (chloroform - methanol) for fractionation to obtain pyroglutamyl-N^{1n}-tert-butoxycarbonyl-histidylprolinamide (15 mg, 35%).
m.p. 155-160°C.
$[\alpha]_D$ = -17.7° (MeOH, C = 0.53).

Continuation from the front page

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